

ASSESSMENT OF PREECLAMPSIA USING ASSAYS FOR FREE AND DISSOCIATED PLACENTAL GROWTH FACTOR

CROSS-REFERENCE STATEMENT

This application is a continuation of International Application No. PCT/US2020/063408, filed Dec. 4, 2020, which claims the benefit of U.S. Provisional Application No. 62/943,739 entitled "Detection Of Preeclampsia Using Assays For Free And Dissociated Placental Growth Factor", filed on Dec. 4, 2019 and U.S. Provisional Application No. 62/947,957 entitled "Detection Of Preeclampsia Using Assays For Free And Dissociated Placental Growth Factor", filed on Dec. 13, 2019, all of which are incorporated by reference herein in their entireties.

BACKGROUND OF THE INVENTION

Preeclampsia is a serious multisystem complication of pregnancy. The incidence of the disorder is generally considered to be between 2% to 8% of all pregnancies, and the disorder carries significant morbidity and mortality risks for both mothers and infants. Preeclampsia is the second largest cause of maternal/fetal deaths and is responsible for approximately twenty billion dollars in healthcare costs annually. In the United States, approximately one million women present with classical symptoms of preeclampsia (hypertension and/or proteinuria after the 20th month of gestation) each year.

The cause(s) and pathogenesis of preeclampsia remain uncertain, and the identification (or ruling out) of preeclampsia using the classical clinical symptoms of the disease is non-ideal. The presentation of classical clinical symptoms can be highly variable, and the symptoms can be indicative of other distinct disorders, such as chronic hypertension, gestational hypertension, temporary high blood pressure, and gestational diabetes. Current laboratories tests (e.g., tests for proteinuria) can be prone to inaccuracies, or are useful for detection of preeclampsia only during relatively late periods in the progression of the disorder. Methods for more reliably predicting whether a pregnant woman will or will not have preeclampsia may, among other things, (1) lead to more timely diagnosis, (2) improve the accuracy of a diagnosis, and/or (3) prevent the unnecessary treatment of women with some preeclampsia symptoms.

Previous studies have used free levels of the vascular factors PIGF and/or sFLT as biomarkers of preeclampsia. However, levels of these free factors alone measured using known methods have limited sensitivity and specificity.

SUMMARY OF THE INVENTION

In some aspects, the present disclosure provides for a method of determining whether a pregnant human female is at risk of suffering from preeclampsia comprising: determining a free Placental Growth Factor to dissociated Placental Growth Factor (PIGF-f:PIGF-d) ratio of a biological sample collected from the pregnant human female, wherein a PIGF-f:PIGF-d ratio less than a first threshold value indicates a heightened risk that the pregnant human female will suffer from preeclampsia, and wherein a PIGF-f:PIGF-d ratio greater than a second threshold value indicates a reduced risk that the pregnant human female will suffer from preeclampsia. In some embodiments, the method comprises collecting the biological sample. In some embodiments, the biological sample is a fluid, whole blood, peripheral blood, serum, plasma, urine, or amniotic fluid sample. In some

embodiments, the method comprises collecting the biological sample via venipuncture, fingerstick sampling, or arterial sampling. In some embodiments, the biological sample is collected from the pregnant woman at gestational week 20 or later. In some embodiments, the biological sample is collected from the pregnant woman prior to gestational week 30. In some embodiments, the risk of developing preeclampsia is the risk of developing preeclampsia within the following one, two, three or more weeks. In some embodiments, the PIGF-f:PIGF-d ratio is determined at least in part by: (a) determining a PIGF-f level by contacting a first PIGF probe to a first portion of the biological sample; and (b) determining a PIGF-d level by contacting the first PIGF probe to a second portion of the biological sample, wherein the first portion of the biological sample is untreated, and wherein a treatment is applied to the second portion of the biological sample, and wherein the treatment dissociates a PIGF complex. In some embodiments, the PIGF complex comprises PIGF and s-FLT. In some embodiments, the treatment is applied for at least 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, or 120 minutes. In some embodiments, the treatment comprises increasing the temperature of the second portion of the biological sample. In some embodiments, the treatment comprises increasing the temperature to at least 30° C., at least 37° C., or at least 45° C. for at least 1 hour or at least 2 hours. In some embodiments, the treatment comprises increasing or decreasing the pH of the second portion of the biological sample. In some embodiments, the treatment comprises contacting the second portion of the biological sample with an agent that prevents PIGF from assembling into a PIGF complex. In some embodiments, the agent is selected from: a surfactant, a detergent, a chaotropic agent, a PIGF-binding protein, and an s-FLT-binding protein. In some embodiments, the first unmodified portion of the biological sample has not been stored at a temperature above about 20° C. In some embodiments, the method has a LLOQ for PIGF of 20 pg/ml or less. In some embodiments, the method comprises (a) determining a level of at least one secondary biomarker selected from total sFLT, free sFLT, Endoglin, KIM-1, FGF21, Decorin, Clec4A and CD274; and (b) determining the pregnant woman as being at risk or not at risk of preeclampsia by application of a trained algorithm to the PIGF-f:PIGF-d ratio in combination with the determined level least one of the secondary biomarker. In some embodiments, the trained algorithm comprises a logistic regression, random forest, nearest centroid, gradient boosting method, linear discriminant analysis, neural network, or support vector machine algorithm. In some embodiments, the level of the at least one secondary biomarker is determined by an immunoassay. In some embodiments, the immunoassay is selected from a capture ELISA, an indirect ELISA, a TR-FRET assay, a proximity extension assay, an amplified luminescent proximity (LOCI) assay, a luminescent oxygen channeling immunoassay, or a lateral flow assay.

In some aspects, the present disclosure provides for a method of treating a pregnant human female comprising: determining whether the pregnant human female has an elevated risk of preeclampsia or reduced risk of preeclampsia by: obtaining a biological sample from the pregnant human female, determining a PIGF-f:PIGF-d ratio in the biological sample, and optionally determining a level of one or more secondary biomarkers in the biological sample, applying a classifier to the PIGF-f:PIGF-d ratio and, optionally, the level of one or more secondary biomarkers to classify the pregnant human female as having an elevated risk of preeclampsia or a reduced risk of preeclampsia; if the